Biopolymer-based materials for biomedical

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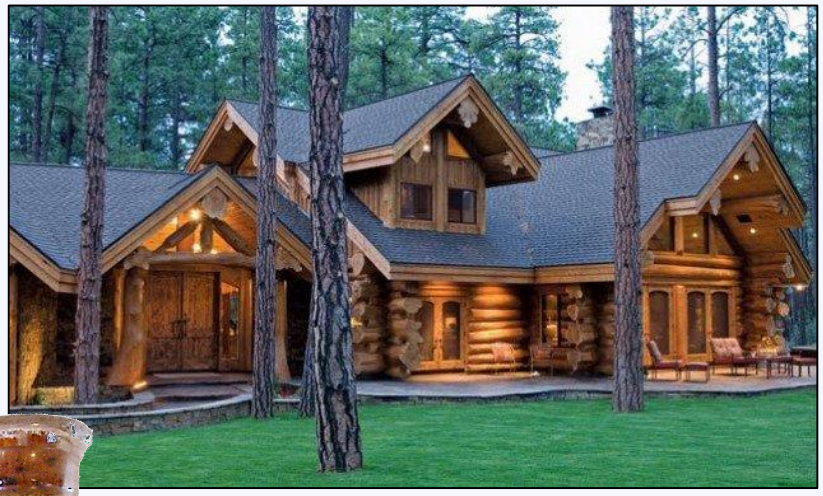
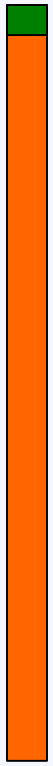
biopolymersforlife.org

Technological applications of plant and animal tissues



Collagen



4 % done!

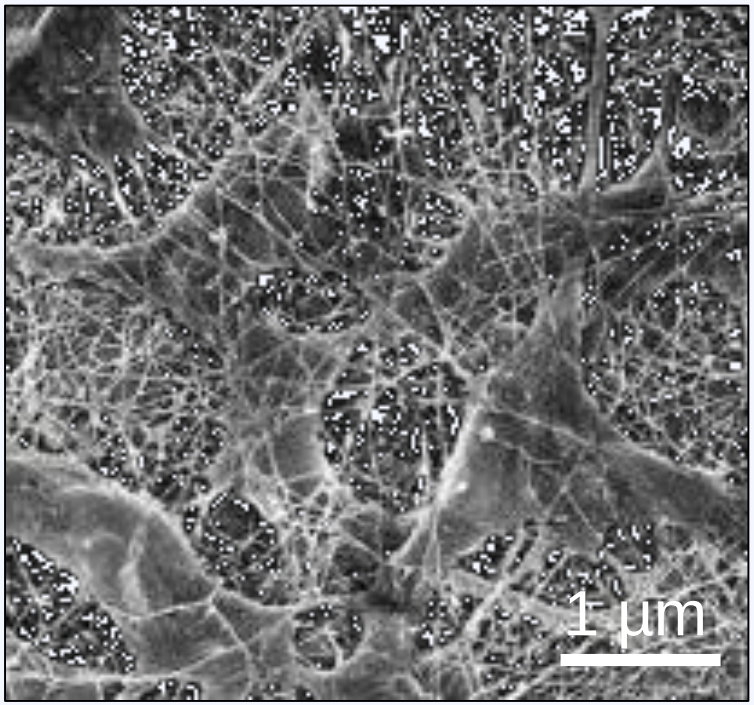
Hyaluronic Acid

Heparin

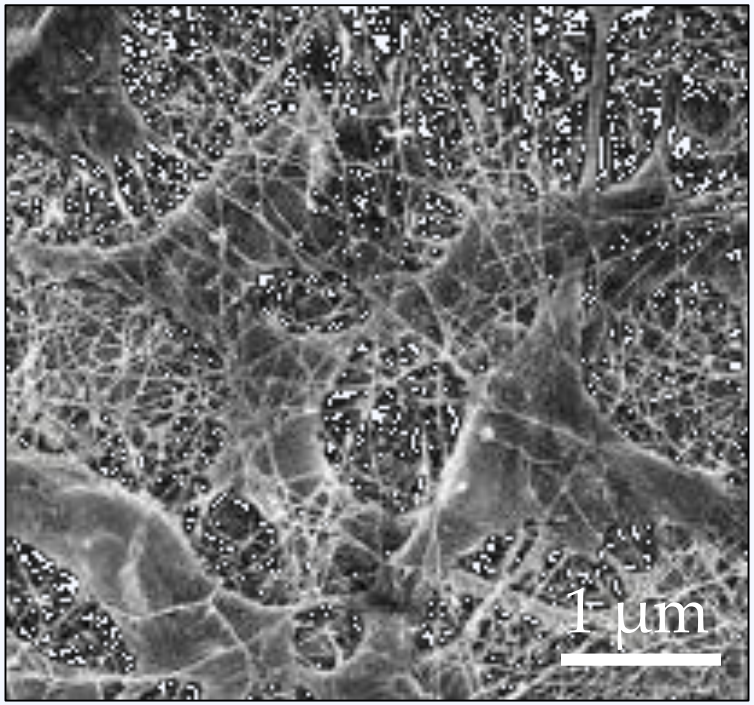
Fibrin

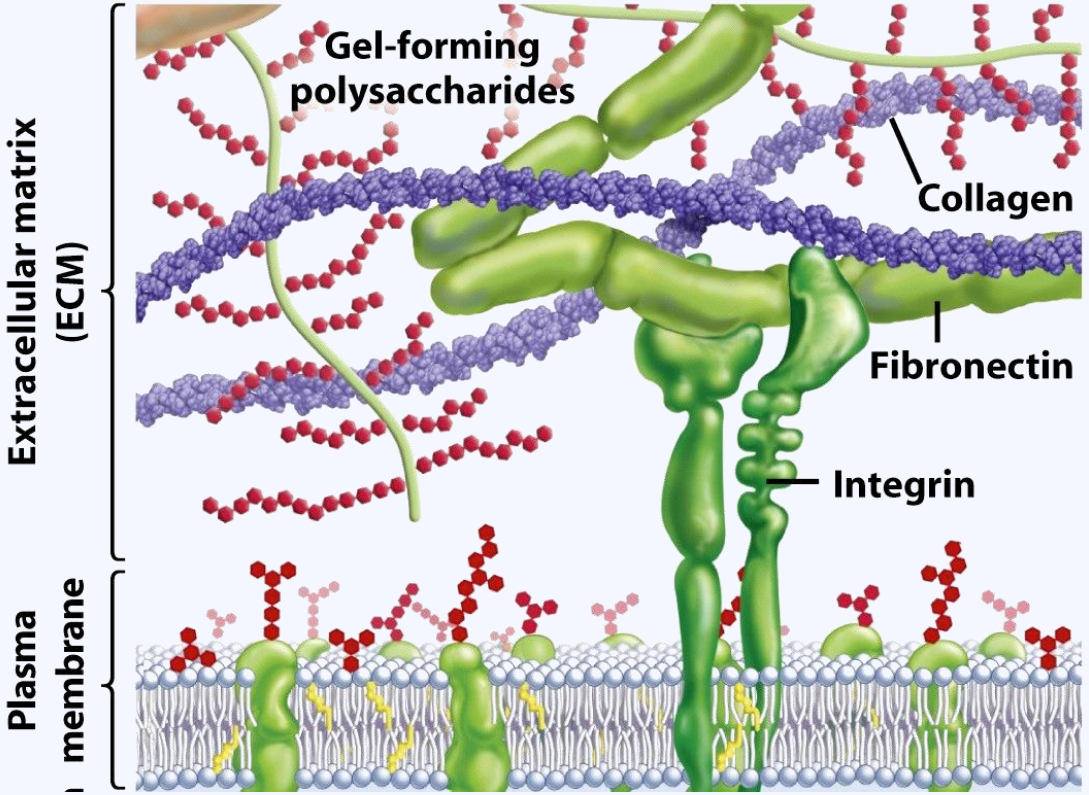
Alginate

Extracellular matrix

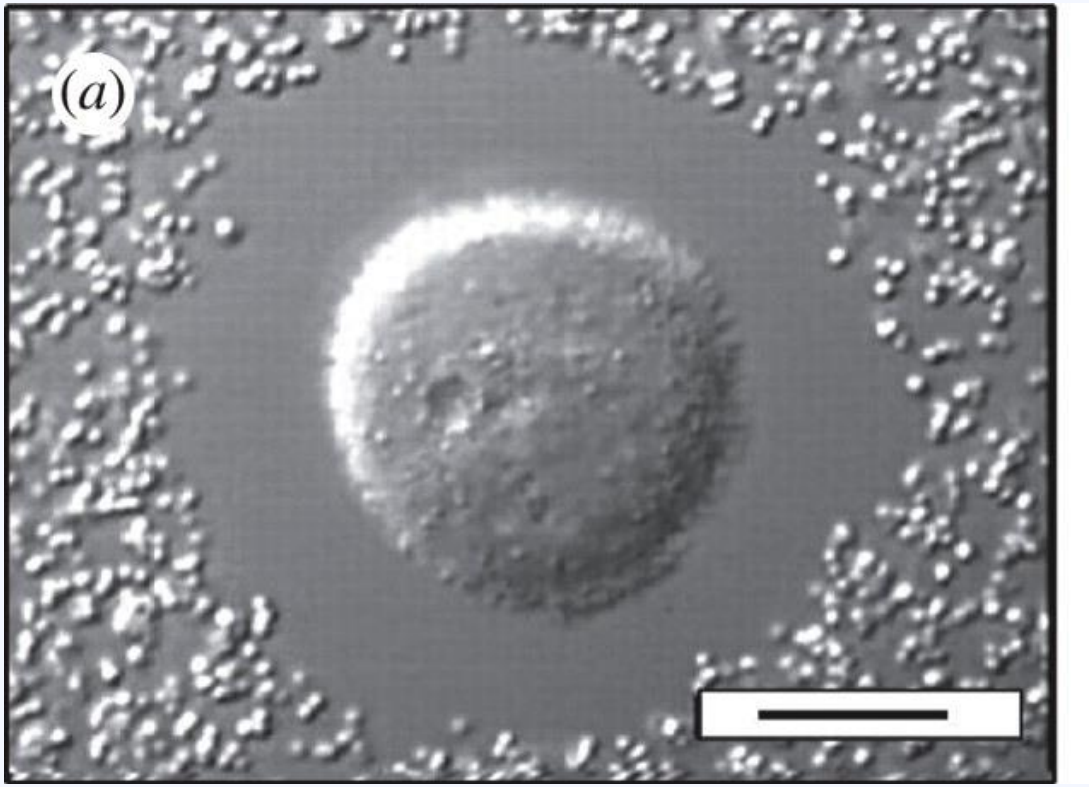


Extracellular matrix





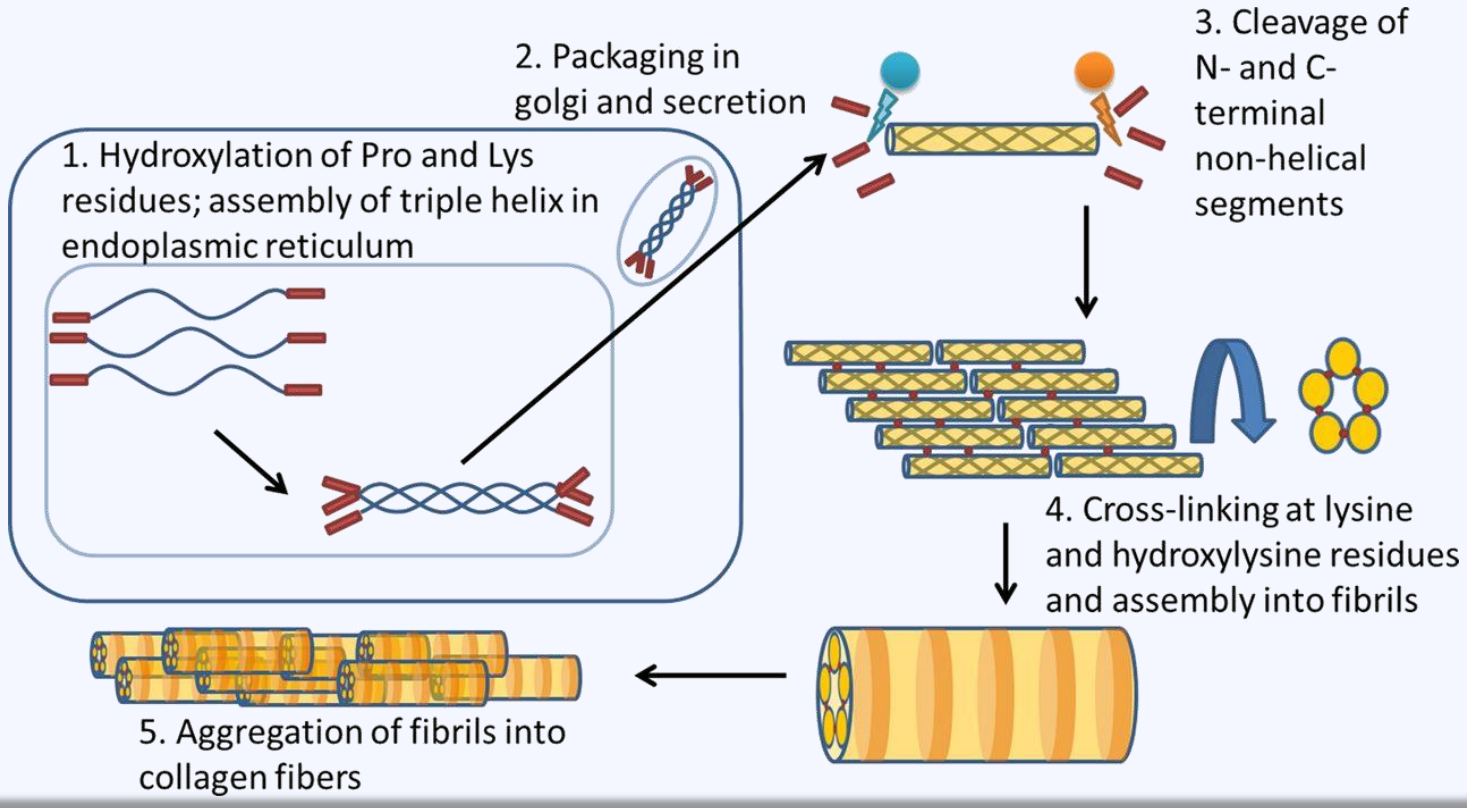
Pericellular matrix



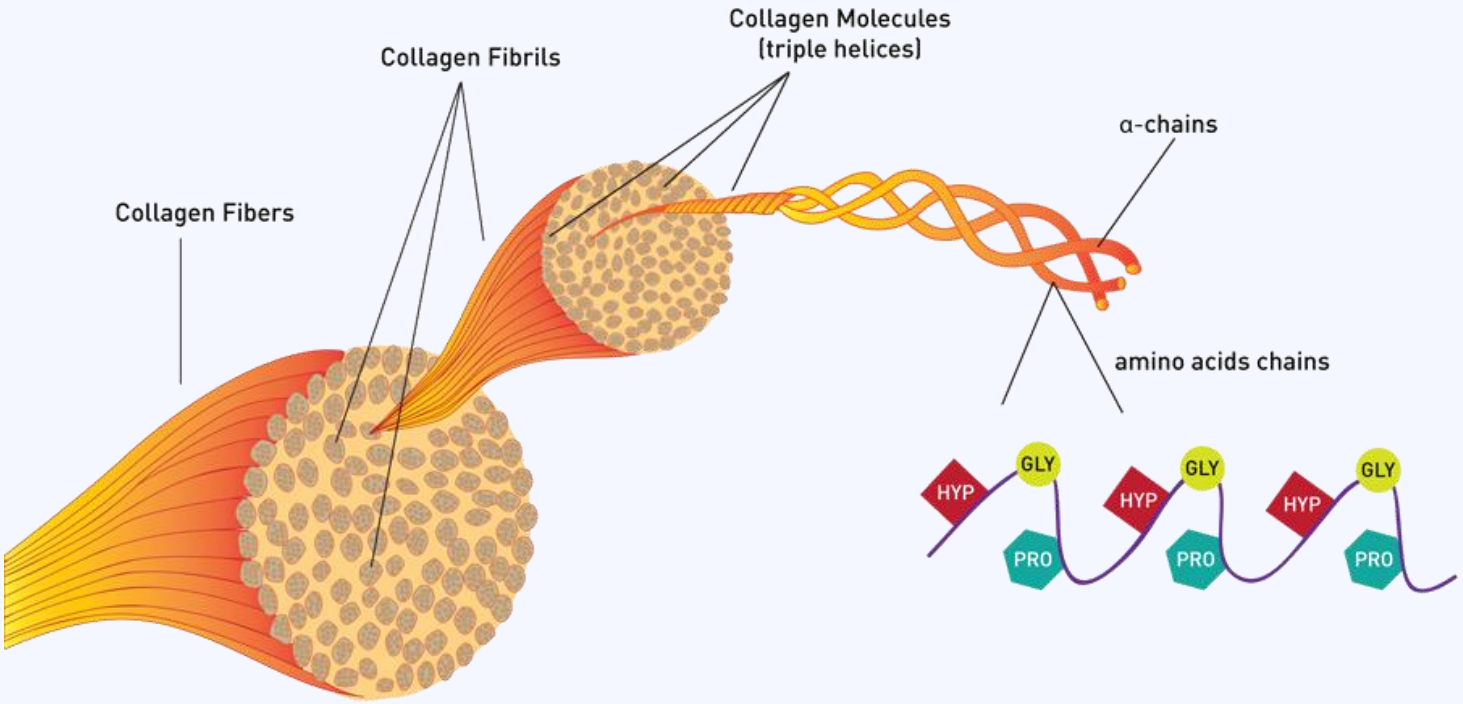
Pericellular matrix (PCM) of PC3 cells

Collagen production

- Greek word: “kola (gum) and “gen” (producing)
- Produced by fibroblasts
- 28 different proteins
- Present in all tissues.

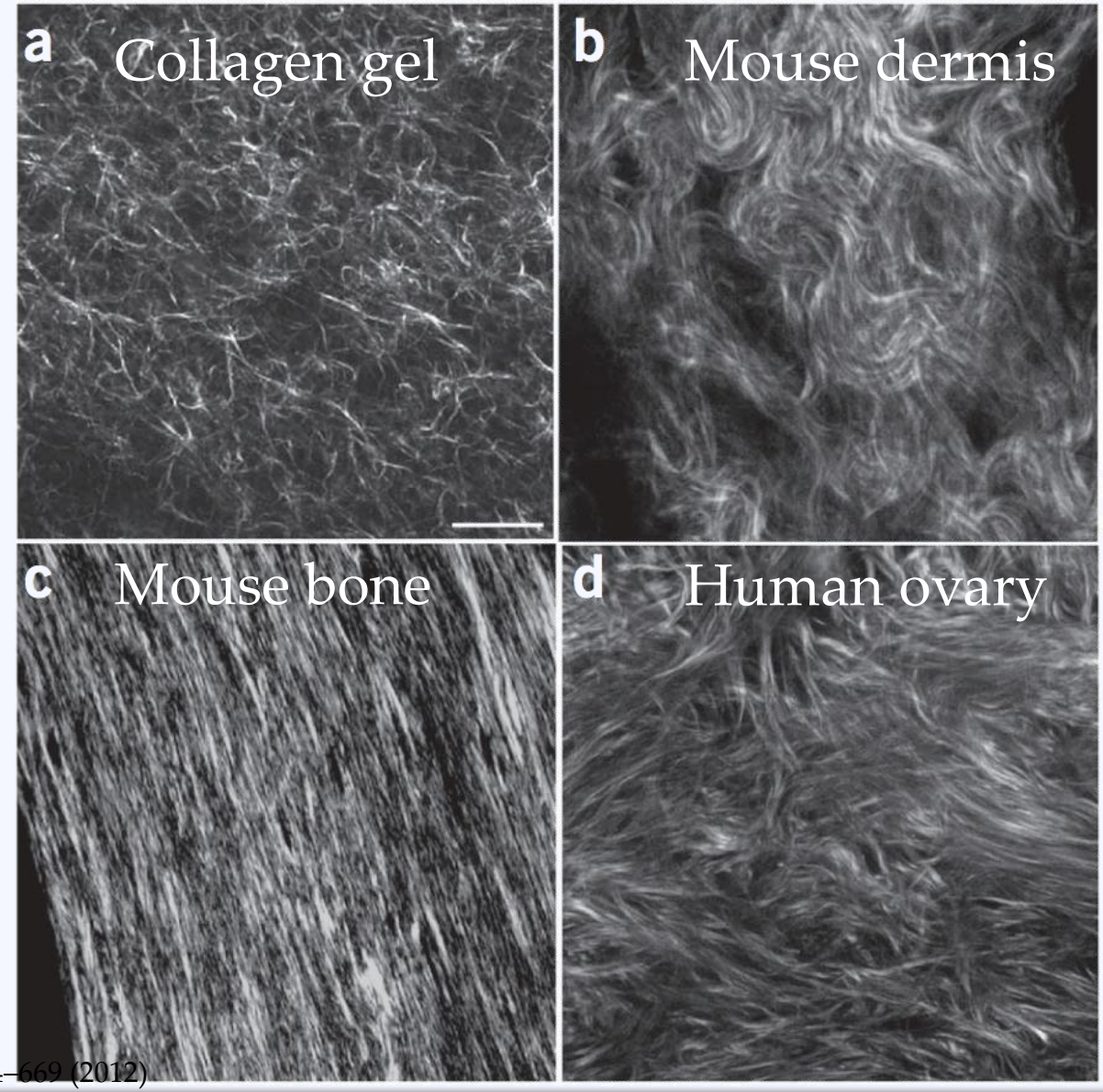


Collagen structure



Gly – X – Y

Collagen structure

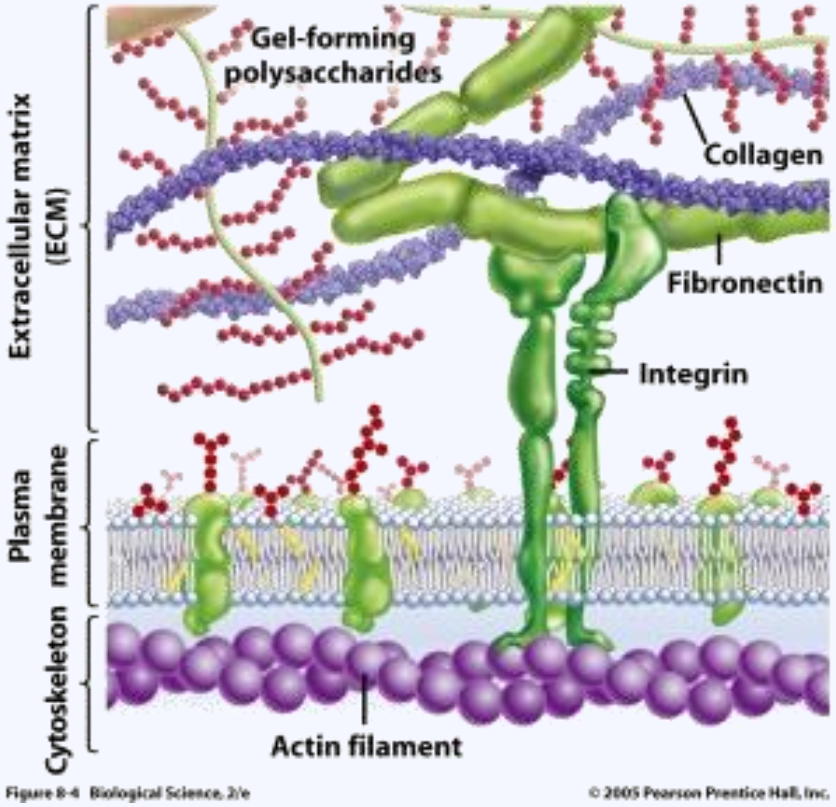


Nature Protocols 7, 654–669 (2012)

Collagen properties

- Structural component of tissues (skin, vessels, eye)
- Binds to signaling molecules
- Degrades into bioactive components

- Involved in many biological processes such as tissue repair.
- Binds to cell receptors (integrins)



Collagen industrial production

<u>Bovine</u>

- Achilles tendon
- placental villi
- nasal or articular cartilage.

<u>Porcine</u>

- dermis
- small intestinal mucosa

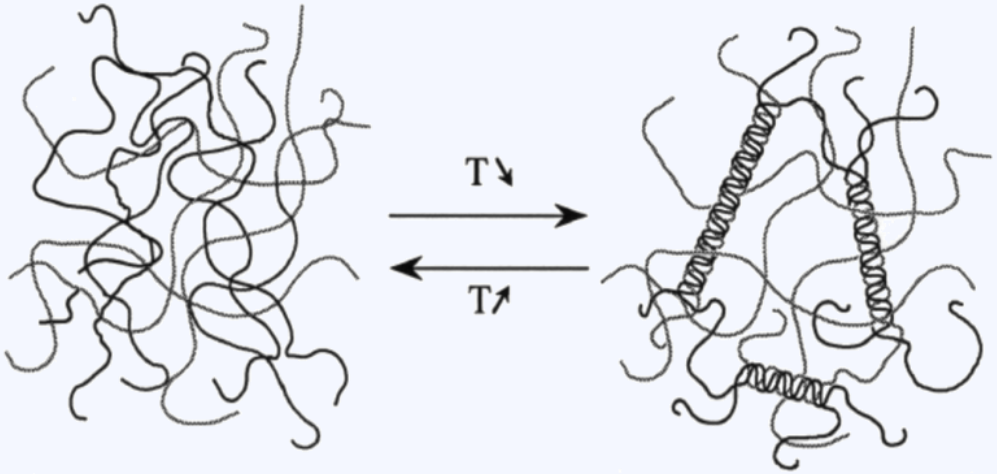
Industrial production

https://www.youtube.com/watch?v=6bYIow9pc6M

Gelatin

What is gelatin?

Collagen hydrolysis by acid, alkali or enzymatic treatment (takes hours to days!)



Collagen properties

- Good mechanical properties
- Takes various forms, injectable gels, membrane, sponges, nano-particles
- Biodegradable
- Bioactive

Global collagen peptide market: **$1.1 billion** in 2012
Global gelatin market**: $3.0 billion** in 2020

"Collagen Peptide and Gelatin Market - Global Industry Analysis, Size, Share, Growth, Trends & Forecast, 2014 - 2020"

What would ***you*** use it for?

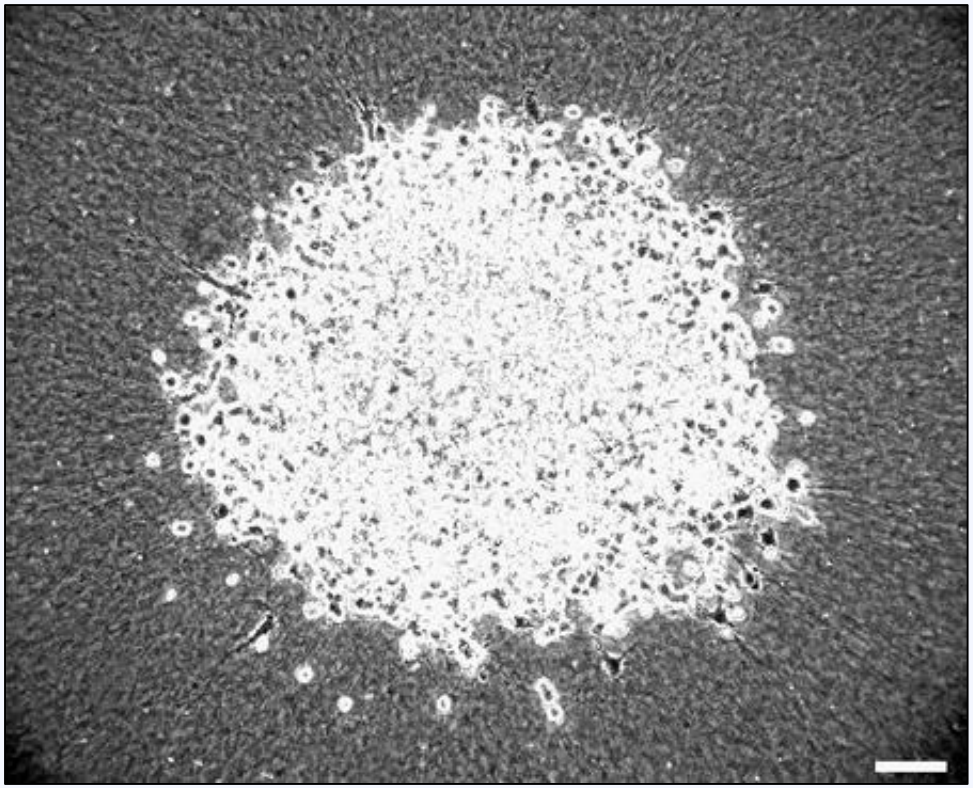
Gelatin Applications

Animal glue

Food industry

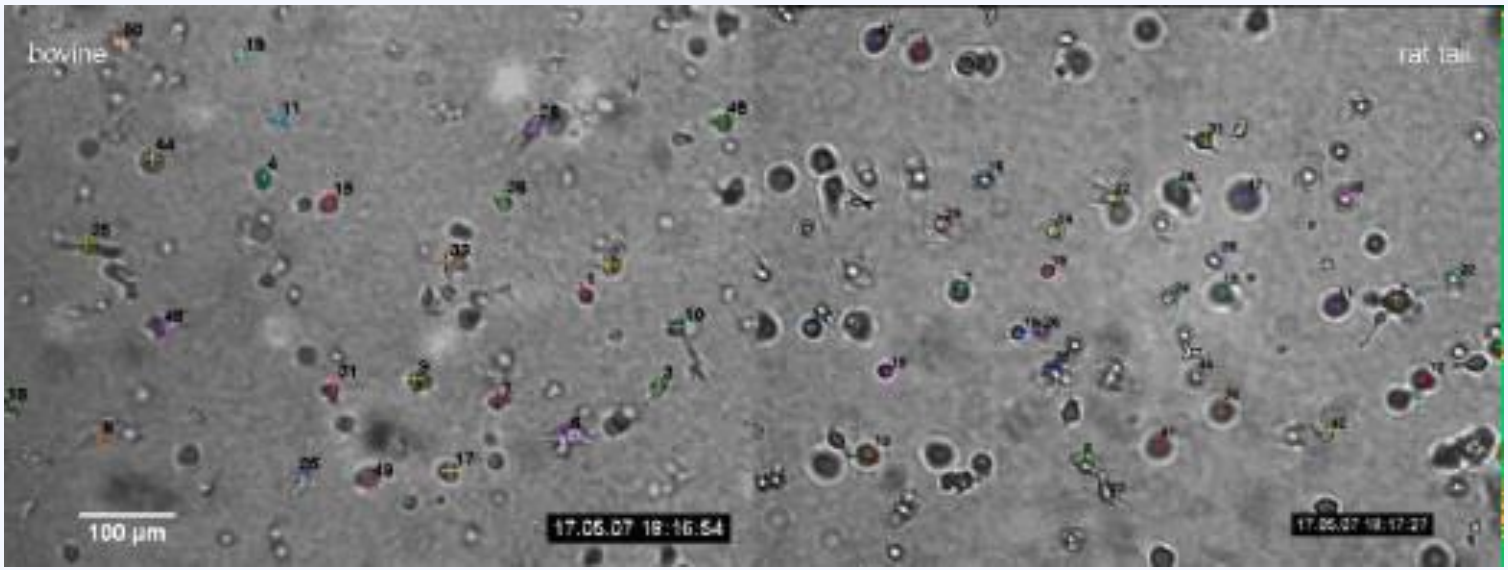
Cosmetic and pharmaceutical industry

Application in biomedical research



Sawhney, R. K. & Howard, J. Slow local movements of collagen fibers by fibroblasts drive the rapid global self-organization of collagen gels. J. Cell Biol. 157, 1083–1091 (2002).

Application in biomedical research

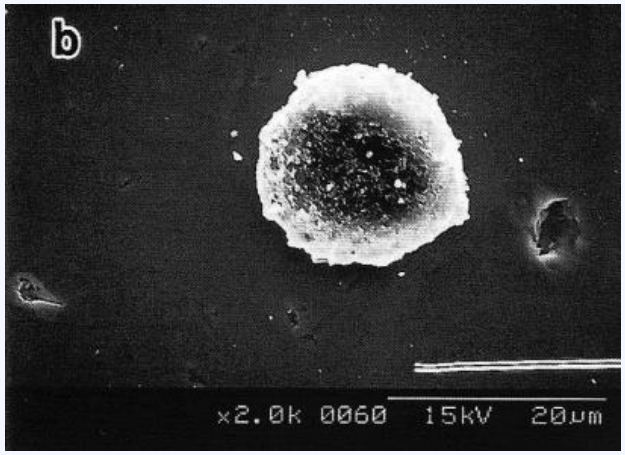


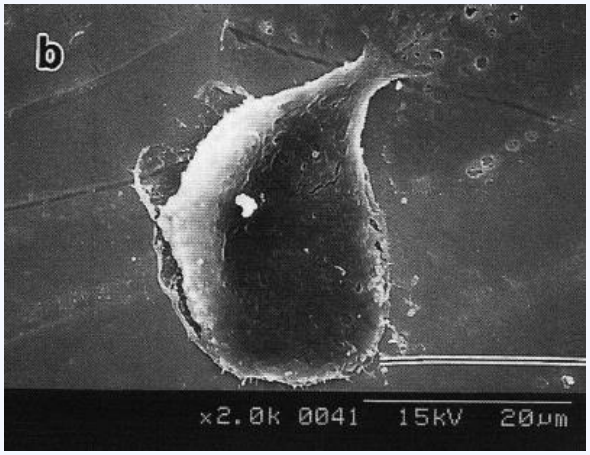
Spontaneous migration of HT1080/MT1 cells, and migration arrest after inhibition of MMP activity by GM6001 in 3D rat tail but not bovine dermal collage

Wolf, K. et al. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. J. Cell Biol. 201, 1069–1084 (2013).

Application in biomedical research

Titanium no collagen Titanium with collagen





Nagai, M. et al. In vitro study of collagen coating of titanium implants for initial cell attachment. Dent. Mater. J. 21, 250–260 (2002).

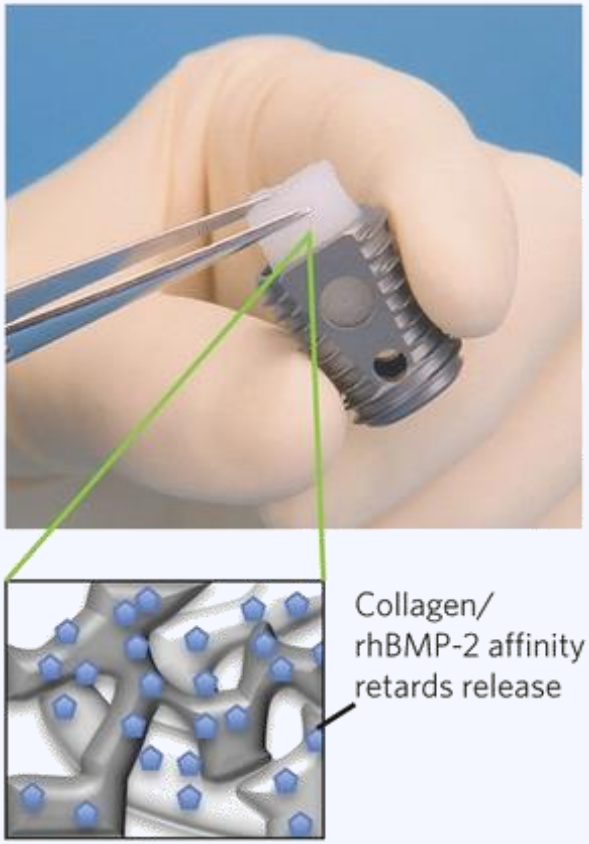
Collagen-based products: wound care

- Problem with wounds: overactive enzymes prevent the formation of the extracellular matrix (ECM)

Collagen based wound dressings:

- Keep enzymes occupied.
- Breakdown products are chemotactic.
- Absorb wound exudates and maintain a moist wound environment.

Collagen-based products: bone regeneration



- Collagen found in bone, involved in bone regeneration.
- Collagen + minerals found in bone: *osteoconductive* properties.
- Collagen + BMP-2 protein to enhance bone generation: *osteoinductive* properties.

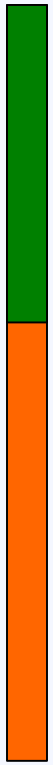
Collagen

Hyaluronic Acid

Heparin

Fibrin

Alginate



42 % done!

Hyaluronic acid (hyaluronan)

Average 70 kg person has 15 grams of hyaluronan in the body

First discovered about 75 years ago:

- In the eye
- In the extracellular matrix
- In the knee joints

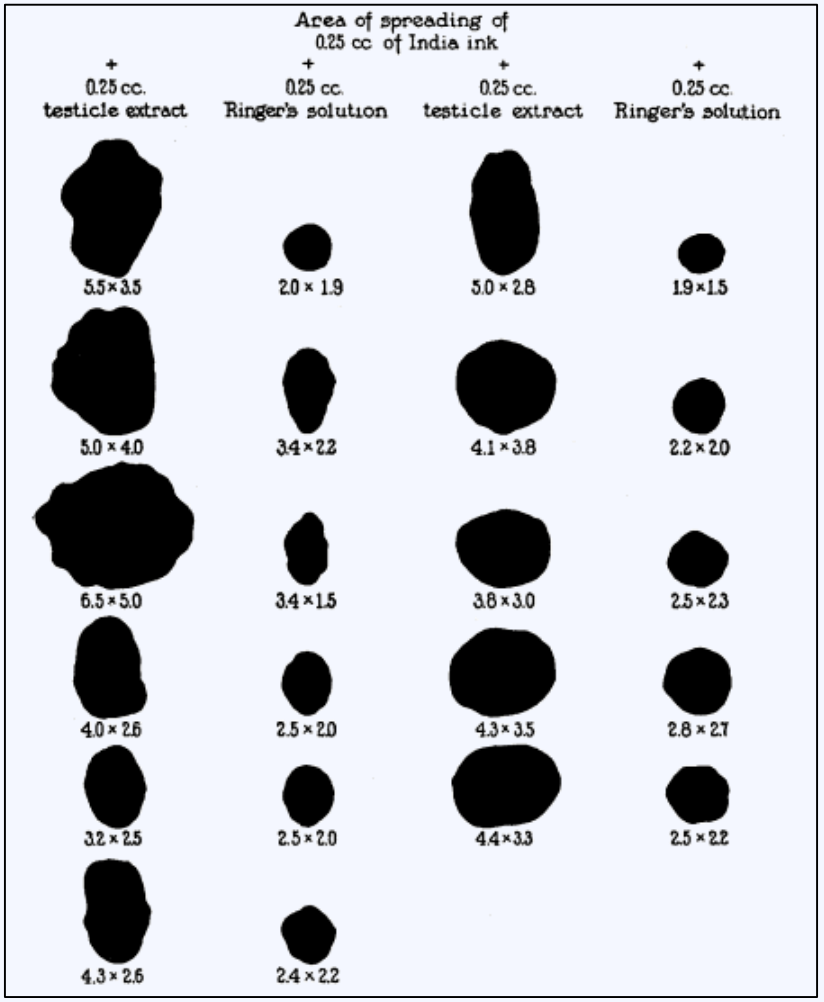
THE INFLUENCE OF TESTICLE EXTRACT ON THE INTRA-DERMAL SPREAD OF INJECTED FLUIDS AND PARTICLES*

By D. C. HOFFMAN, M.D. AND F. DURAN-REYNALS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

PLATE 18

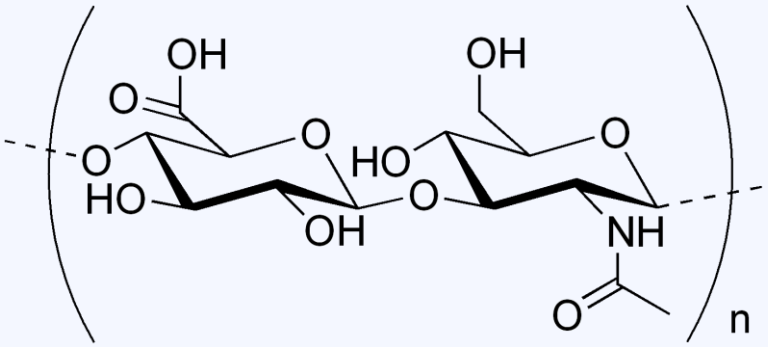
(Received for publication, November 13, 1930)



Why would the ink spread more when co-injected with testicle extract?

Hyaluronic acid structure

1950s - Karl Meyer and his team made great advanced to understand HA structure.

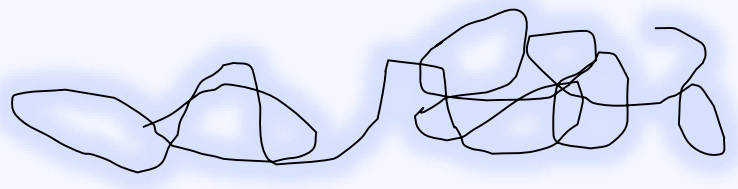


Polymer of disaccharides composed of:

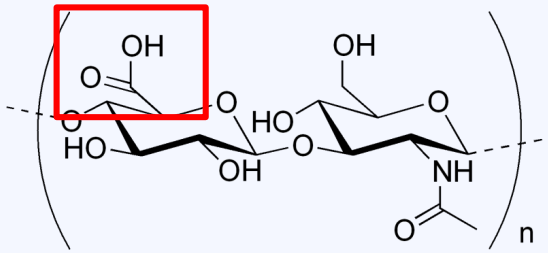
- D-glucuronic acid and
- D-N-acetylglucosamine
- Linked via alternating β-1,4 and β-1,3 glycosidic bonds.

Structure function relationship

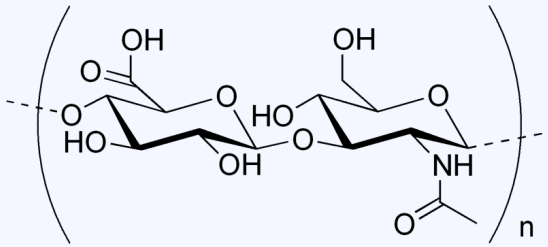
High molecular weight



Negative charge (carboxylic acid)



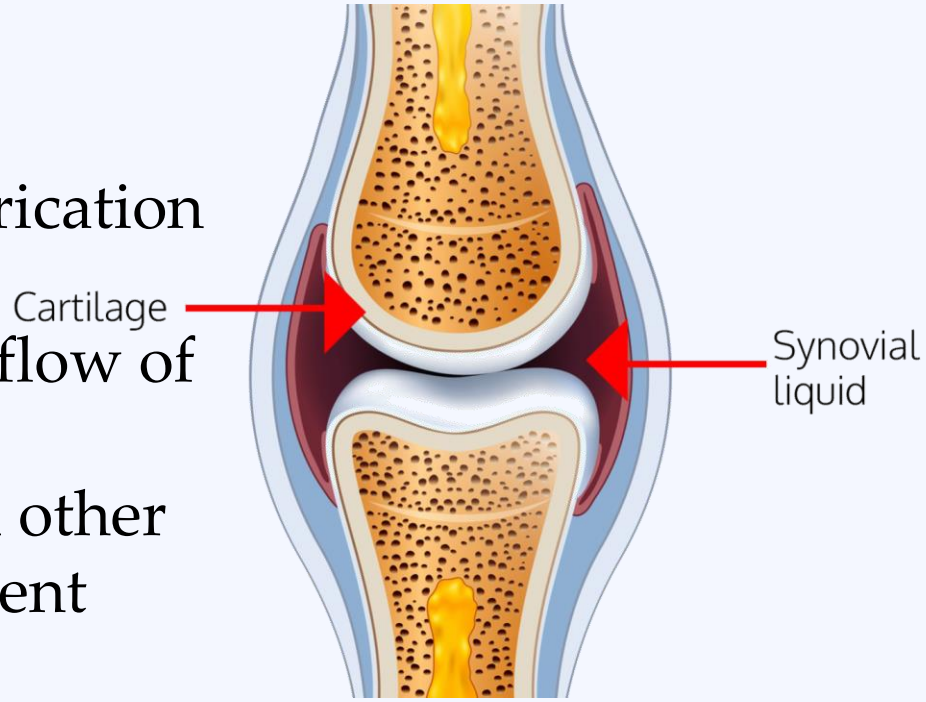
High hydration (hydrogen bonds)



What properties would you expect?

Hyaluronic acid functions

- Rheology (shear dependent viscosity), important for lubrication of joints and tissues.
- Water homeostasis: prevent flow of water through tissues
- Barrier effect / interacts with other extracellular matrix component (Collagen type VI)

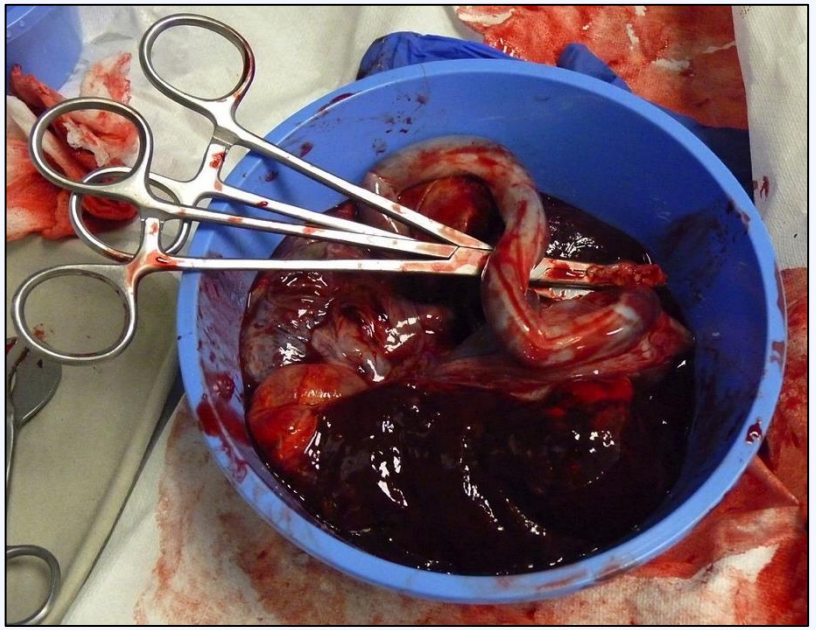


- Cell signaling (CD44 receptor)
  - Triggers cancer metastasis
  - Protects cells during migration
- Also involved in wound repair, inflammation, cell migration…

Hyaluronic acid applications

In 1943 N.F. Gamaleya created bandages to treat the frostbites.

The main component of the bandage was an extract from the umbilical cord.



The USSR Ministry of Health and the drug received the name *"Regenerator"*.

Human umbilical cord contains a significant amount of HA (major industrial source at the time)

Industrial production

Since the early 1930s when HA was first isolated from bovine vitreous humora

"the yield is between 0.7 and 1.0 gm. per 100 eyes."
(Meyer and Palmer 1934)

HA extraction has been widely carried out using other animal tissues including human umbilical cord, rooster comb, and bovine synovial fluid.

Industrial production

Why move away from extraction?

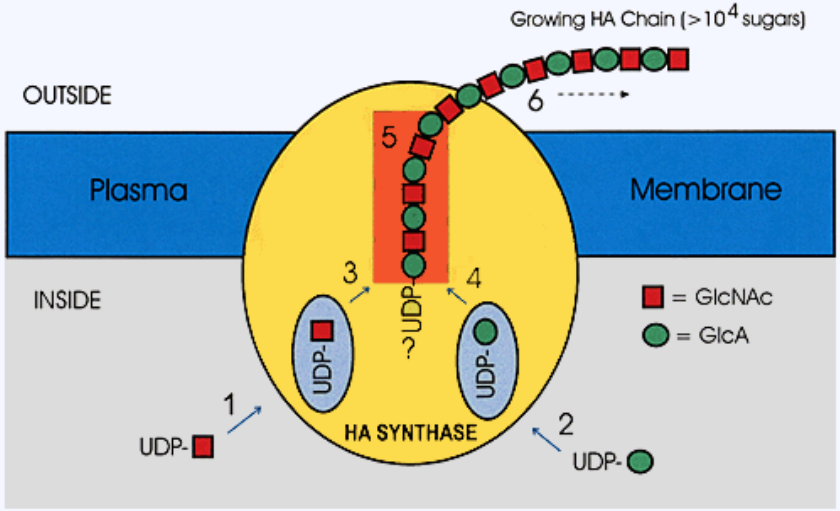
- grinding, acid treatment, and repeated extraction with organic solvents. Affects yield and polydispersity (range of sizes)
- bound to cellular proteins (hyaluronidase)
- contamination with nucleic acids, prions (bovine) and viruses (avian) which could result in the transmission of infectious diseases
- costly since labor intensive and requires large facilities that can accommodate processes involved from collection of tissue from the animal to extraction and purification.

Industrial production

- **B. subtilis** has been successful at producing HA.
  - High capacity to grow in industrial fermenters
  - Exotoxins and endotoxins free
  - Products awarded the GRAS (generally recognized as safe) designation
- **E. coli also produces HA** if supplemented with glucose and glucosamine

Hyaluronic acid synthesis

Hyaluronan synthases (HAS)



Multiple Functions of Hyaluronan Synthases

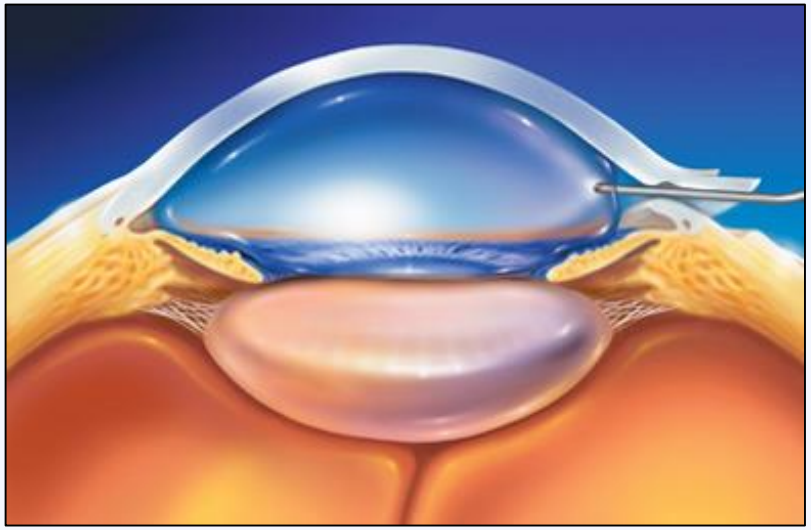
1) UDP-GlcNAc Binding Site
2) UDP-GlcA Binding Site
3) beta (1,4) GlcNAc Transferase
4) beta (1,3) GlcA Transferase
5) HA (acceptor) Binding Site
6) HA Transfer (translocation)

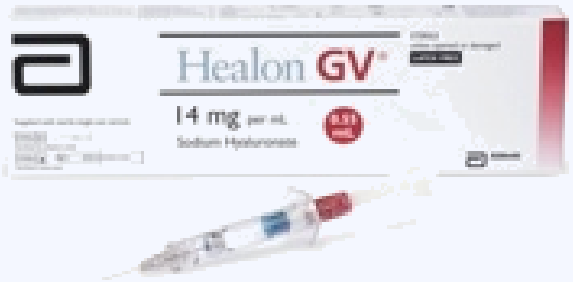
The hyaluronan capsule of Group C Streptococcus cells (Scale is at 1 μm)

Hyaluronic acid applications

Patents “hylauronic acid” = 80 000
World market: **$2.5 billion** in 2017

First biomedical product: Healon (1980s by Pharmacia) for eye surgery





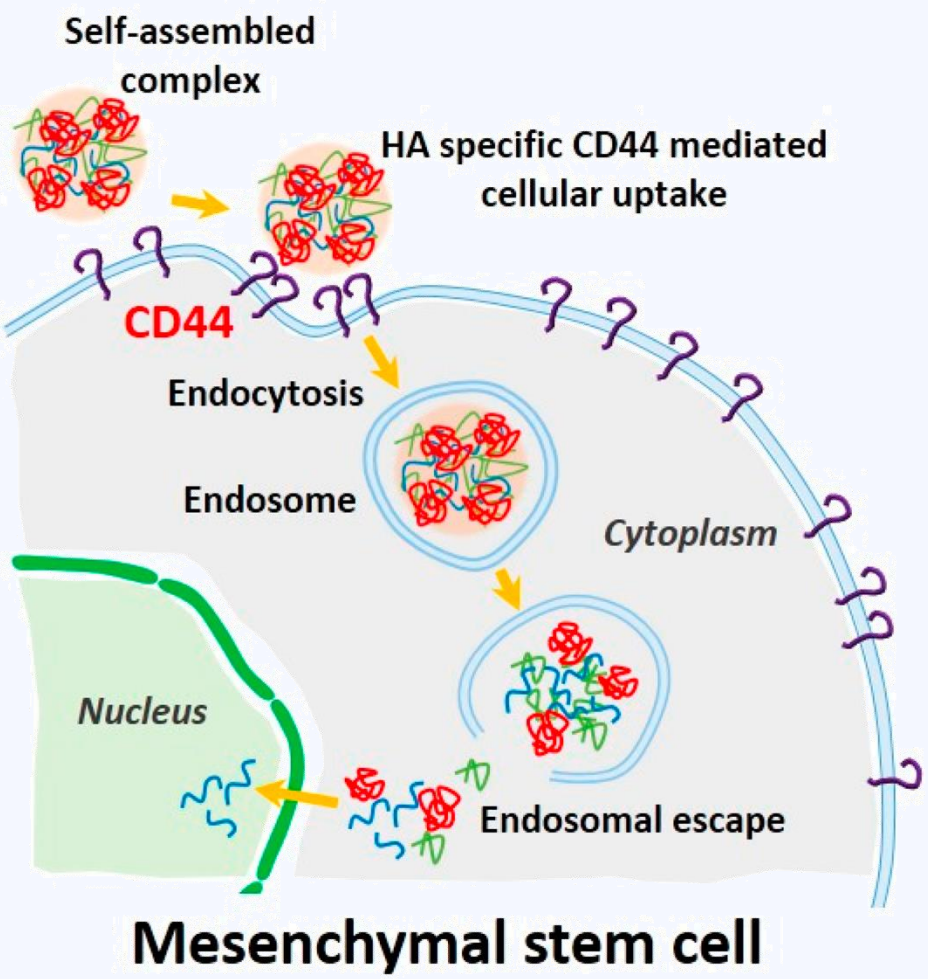
Hyaluronic acid applications

- Drug delivery systems
- Tissue engineering and repair (in ophtamology, joints, aesthetic medicine
- Cosmetics

Hyaluronic acid applications

HA use in drug delivery:

- HA can be modified chemically to increase interactions with drugs
- HA binds to CD44 cell surface receptor and triggers cellular uptake
- Good biocompatibility and biodegradability of HA



Molecules 2016, 21(5), 572

47% done!

Collagen

Hyaluronic Acid

Heparin

Fibrin

Alginate

Heparin

- Team of William Henry Howell in Baltimore looking for coagulation factors and anticoagulation factors.
- Extracting molecules from brain, liver.
- Liver-extracted molecules seems to be anticoagulant: hepa- (liver in greek) rin.
- By 1920, produced in US Baltimore although not pure

Heparin

CCXIII. THE CHEMISTRY OF HEPARIN.

By ERIK JORPES.

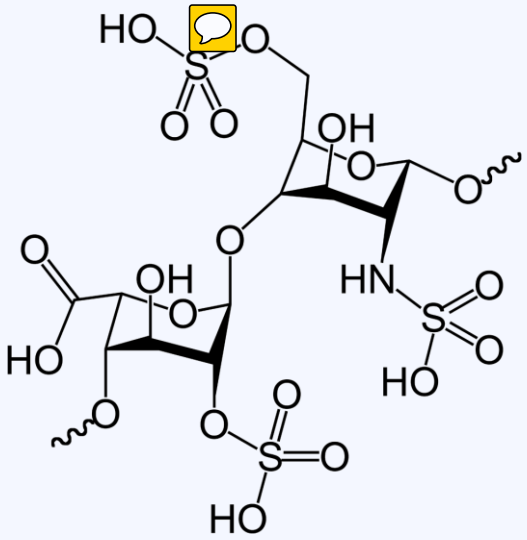
From the Physiological Chemistry Department of the Caroline Institute, Stockholm, and the Insulin Laboratory of the Vitrum Company, Stockholm.

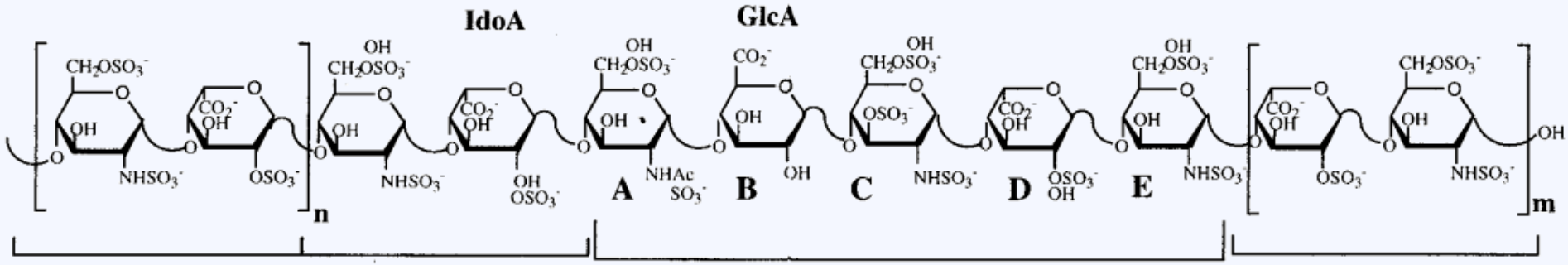
(*Received May 13th, 1935.*)

HEPARIN, the anticoagulant discovered by Howell [1918], has not until recent times been readily accessible either to the research chemist or to the physiologist. Its high price has prevented a thorough study of its chemical properties and its more extensive use in physiological laboratories and in clinical practice. Thanks to the recent work of Charles and Scott [1933], fresh possibilities have been afforded in both directions. Heparin has been shown to be a common tissue constituent, and the method of its preparation has been greatly improved.

Heparin

- N-acetyl-D-glucosamine (GlcNAc)
- Glucuronic acid (GlcA)
- Sulfation at different positions

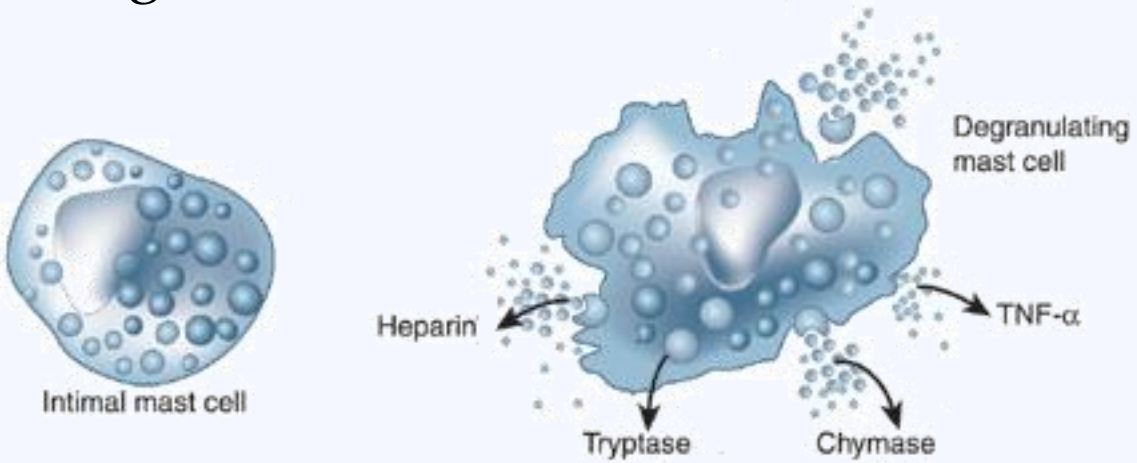




n + m = 16
for MW 12,000

Heparin synthesis and function

- Heparin is production mast cells (tissue resident cells part of immune system)
- Found in lung and liver.



Role of heparin in the body is not totally clear:

- Anticoagulation
- Defense against pathogen

Anticoagulant activity

- Heparin binds to the enzyme inhibitor antithrombin III
- Causes a conformational change that inactivates thrombin and other proteases involved in blood clotting
- Thrombin serine protease that converts soluble fibrinogen into insoluble strands of fibrin

Heparin production

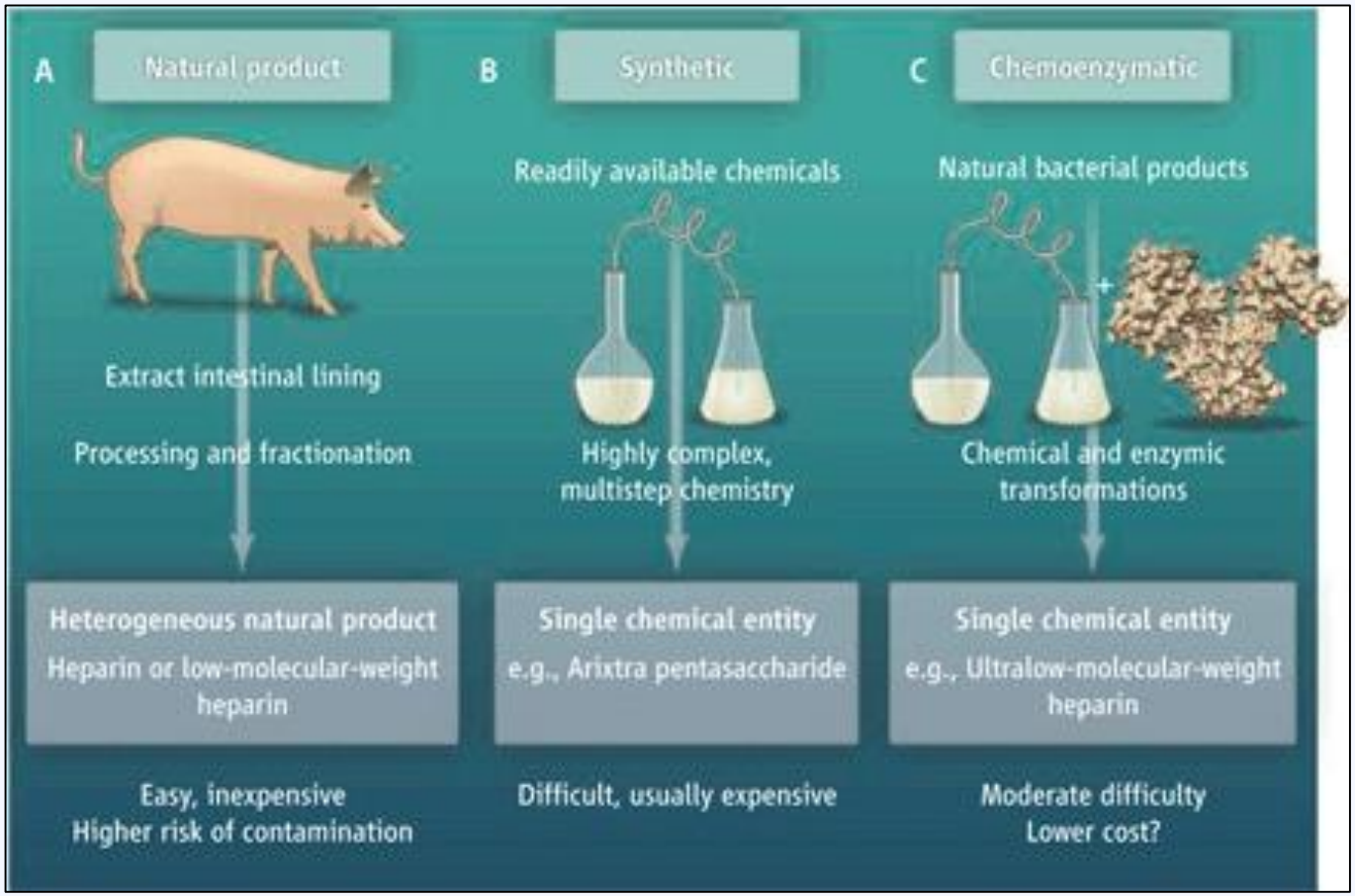
Medical grade produced using animals tissues such as porcine (pig) intestines or bovine (cattle) lungs.

During 2007 and 2008, a cluster of serious adverse events occurred that were caused by contamination of some heparin supplies with over-sulfated polysaccharides (chondroitin sulfate).

Their administration resulted in more than 200 deaths in the United States alone, apparently in most cases from allergic or hypersensitivity-type reactions.

What would you do?

Heparin production

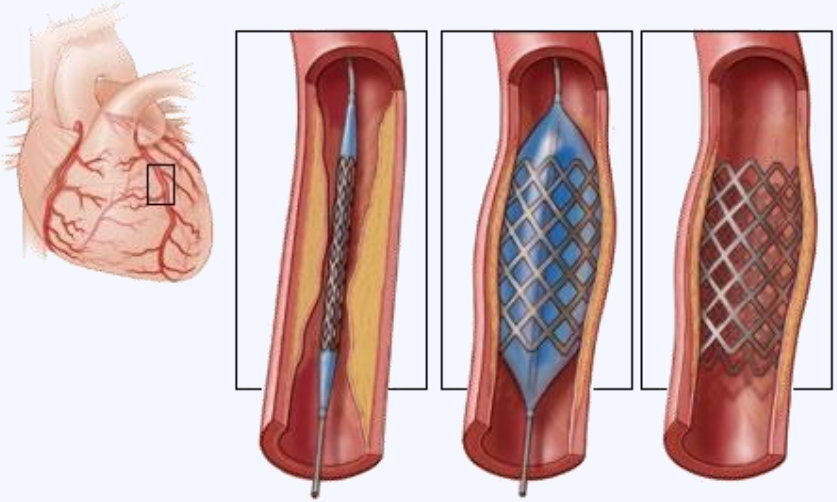


Turnbull, J. E. Chemistry. Getting the farm out of pharma for heparin production. Science 334, 462–463 (2011).

Heparin Applications

A $2 billion market in 2013

- Injections (prevent blood clots in arteries veins, lung) or before surgery.
- Coating of syringe and other implanted materials.



Carmeda® BioActive Surface

Collagen

Hyaluronic Acid

Heparin

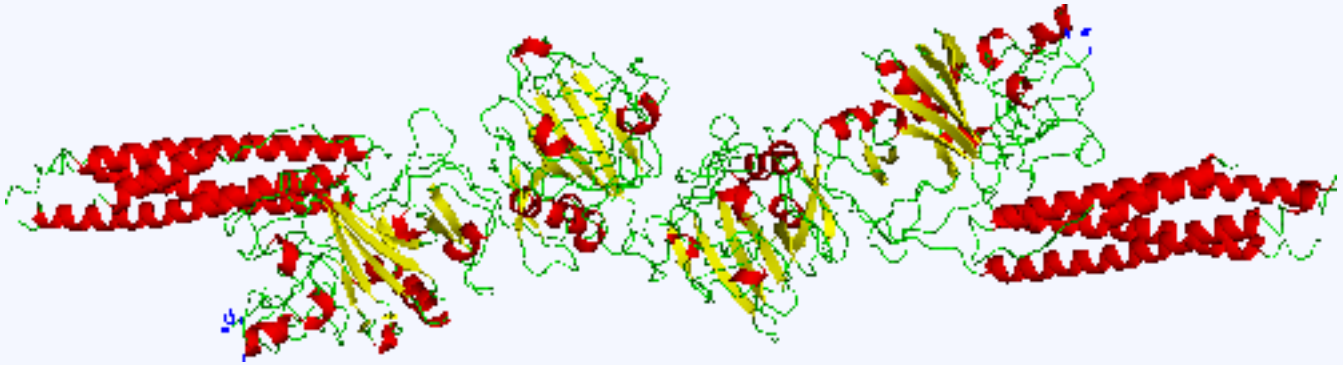
Fibrin

Alginate

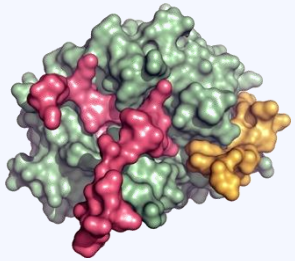
59 % done!

Fibrinogen and thrombin

Fibrinogen (340 kDa plasma glycoprotein)



Thrombin



Formation of fibrin

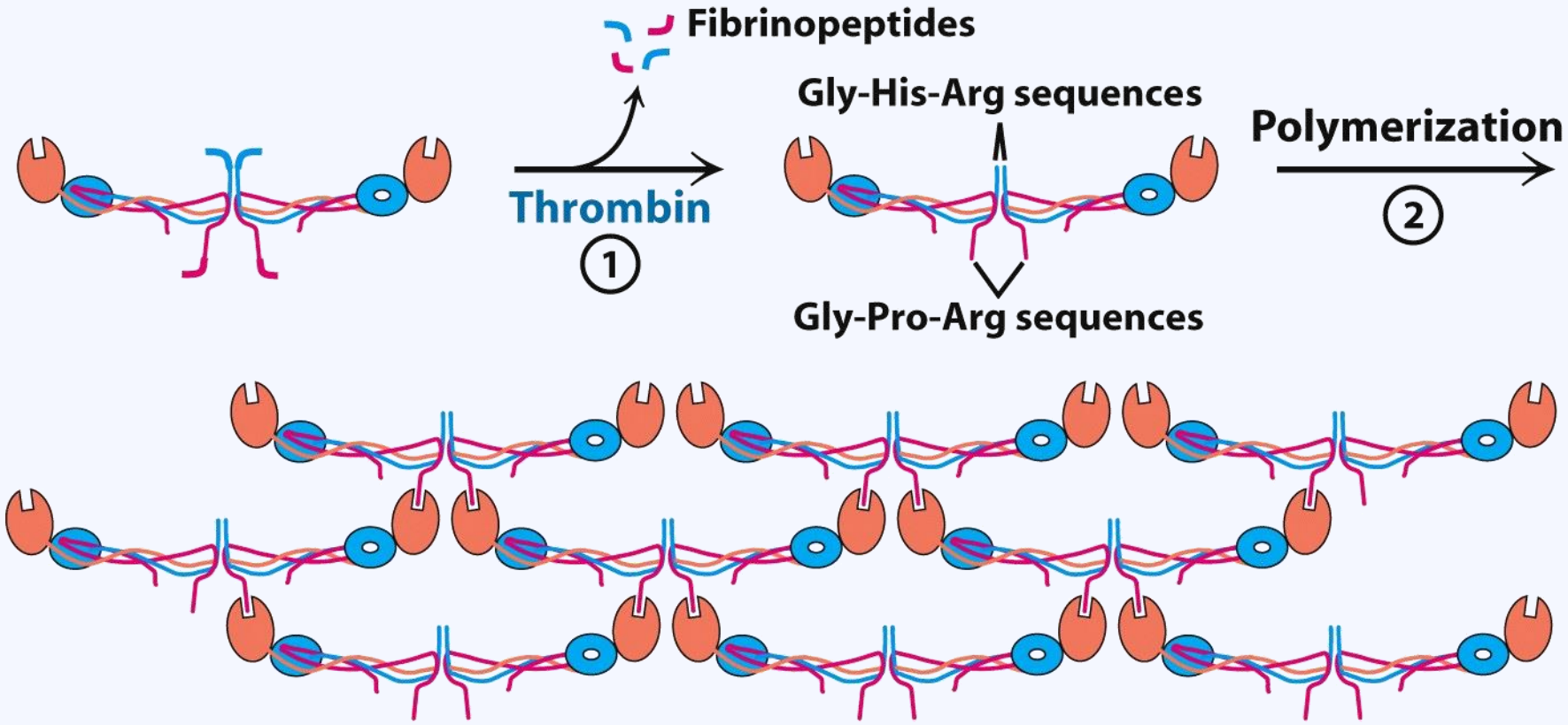


Figure 10.29
***Biochemistry*, Seventh Edition**

Interactions between knobs ‘A’ and holes ‘a’.
Strong and stable

Fibrin network

Fibrinogen synthesis

Fibrinogen is synthesized in the liver by the hepatocytes

- 200 to 300 mg / 100 ml plasma.
- Following tissue injury, level can increase, 2- to 10-fold
- Chain assembly is rapid: 6-chain molecule assembled < 5 min

Historical use of fibrin

In the 1940s. Crude systems containing fibrinogen and thrombin were utilized.

In the 1960s. Improved product with development of fibrinogen purification techniques.

Used for

- wound healing
- skin grafting
- surgical sealing
- provide hemostasis in microvascular surgery
- serve as a matrix for bony chips and fragments in the repair of bone defects

Cohn fractionation

Based on the variation of

- pH
- ethanol concentration
- temperature
- ionic strength
- protein concentration

How are these parameters impacting fibrinogen?

Differential solubility of the various plasma proteins

Cryo-precipitation

Protocol:

1. -20 ° C or less for at least 12 hours.
2. slowly thawed at 4 ° C
3. centrifugation to isolate the fibrinogen precipitate.

Fibrinogen obtained using this method has a final concentration of 6 mg/ml and lower clottability due to large amount of fibrinogen being denatured.

Fibrinogen production

Batch size: 2000-4000L

Thierry Burnouf, PhD

Problems…

In 1978, the FDA revoked the license for commercial fibrinogen concentrates, the use of which was causing high rates of hepatitis.

What was the origin of the hepatitis?
How would you solve it?

Removing viral contamination

Three major methods to ensure viral safety:

1. Avoidance = biopolymer is produced in a non-animal organisms

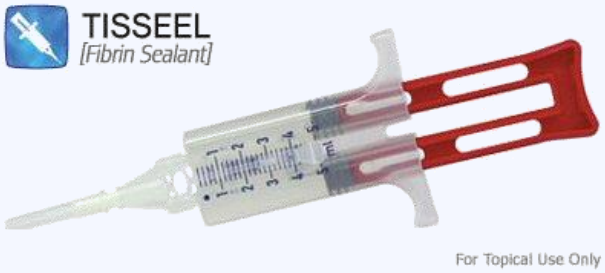
2. Inactivation = virus destruction/kill by alteration of its capacity to replicate

3. Removal = partitioning of viruses and plasma proteins in different fractions

Removing viral contamination

Source	Human fibrinogen (mg/mL)	Virus inactivation
Immuno (Austria)	90	Two-step vapor heat
Behringwerke (Germany)	65-110	Wet heat, 10 hours, 60°C
CRTS (France)	90	SD†
SNBTS (Scotland)	40	Dry heat, 72 hours, 80°C
Haemacure Biotech (Canada)	50-70	SD, nanofiltration, dry heat, 100°C, 2 hours
Baxter/American Red Cross	100	SD
NYBC	150	SD and UVC‡

Fibrin commercial products



Commercially available fibrin sealant was approved in Europe in 1982.

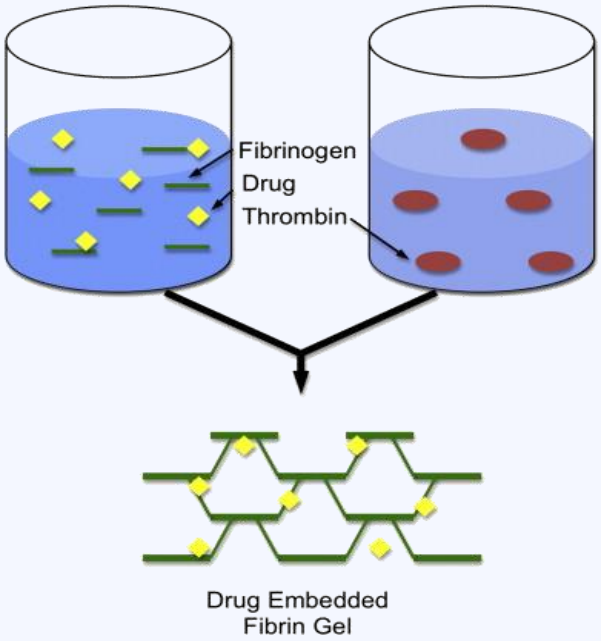
In 1998, Tisseel and Hemaseel fibrin sealants in the USA.

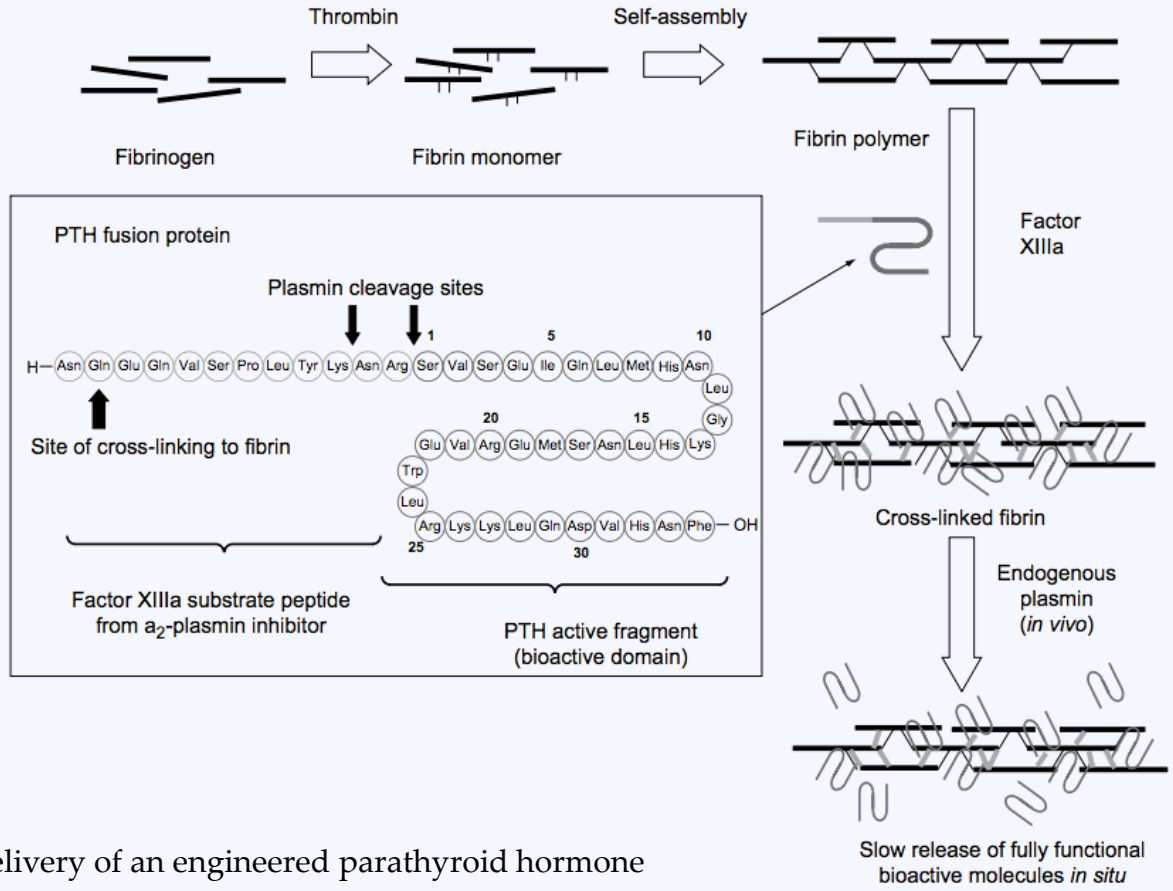
In 1990s, American surgeons use noncommercial forms of fibrin sealant (combining patient's own plasma clotting factors through autologous donation, with bovine thrombin).

Fibrin use in surgery

Future use of fibrin

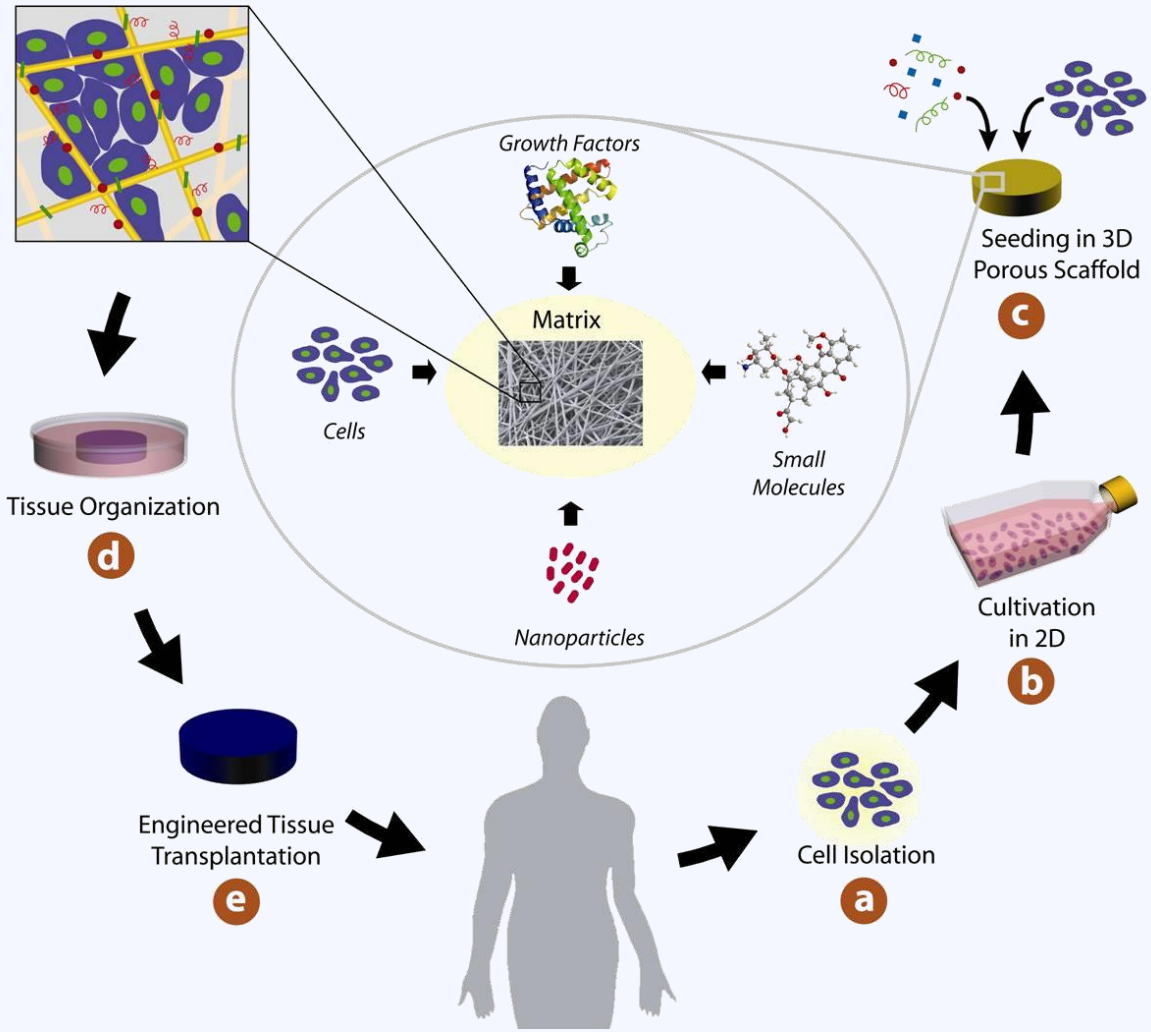
Drug delivery





Arrighi, I. et al. Bone healing induced by local delivery of an engineered parathyroid hormone prodrug. Biomaterials 30, 1763–1771 (2009).

Future use of fibrin



http://www.tau.ac.il/lifesci/departments/biotech/members/dvir/images/Picture1Dvir.jpg

80 % done!

Collagen

Hyaluronic Acid

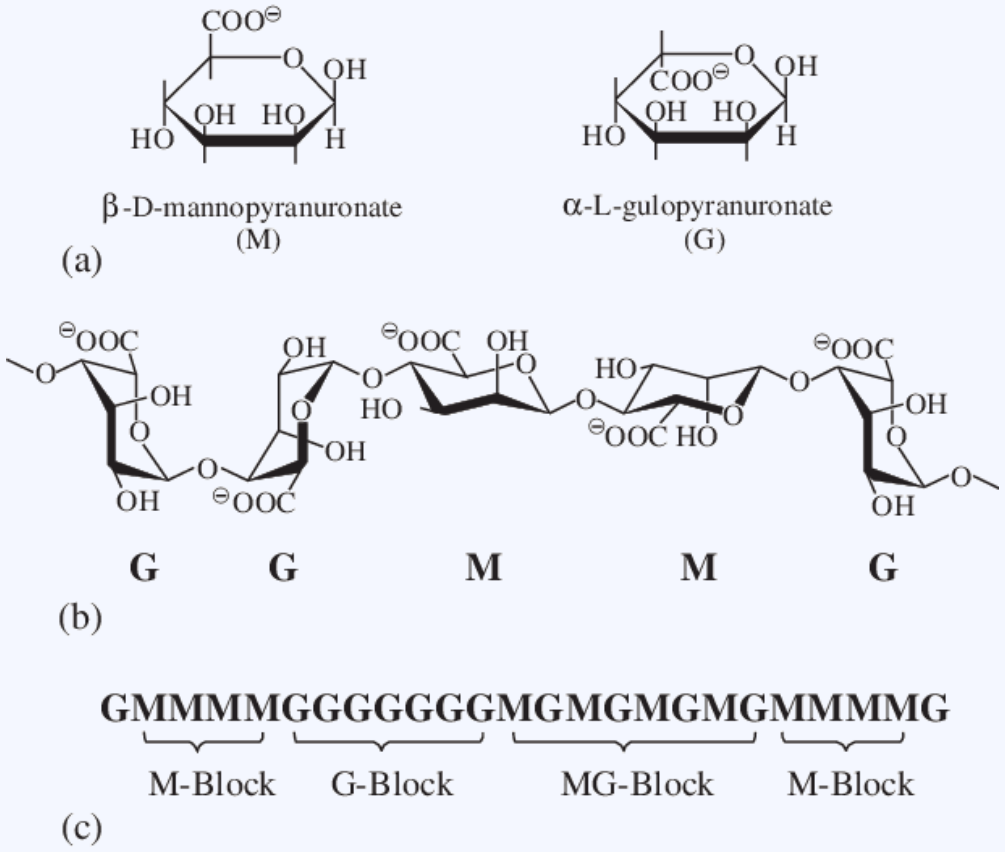
Heparin

Fibrin

Alginate

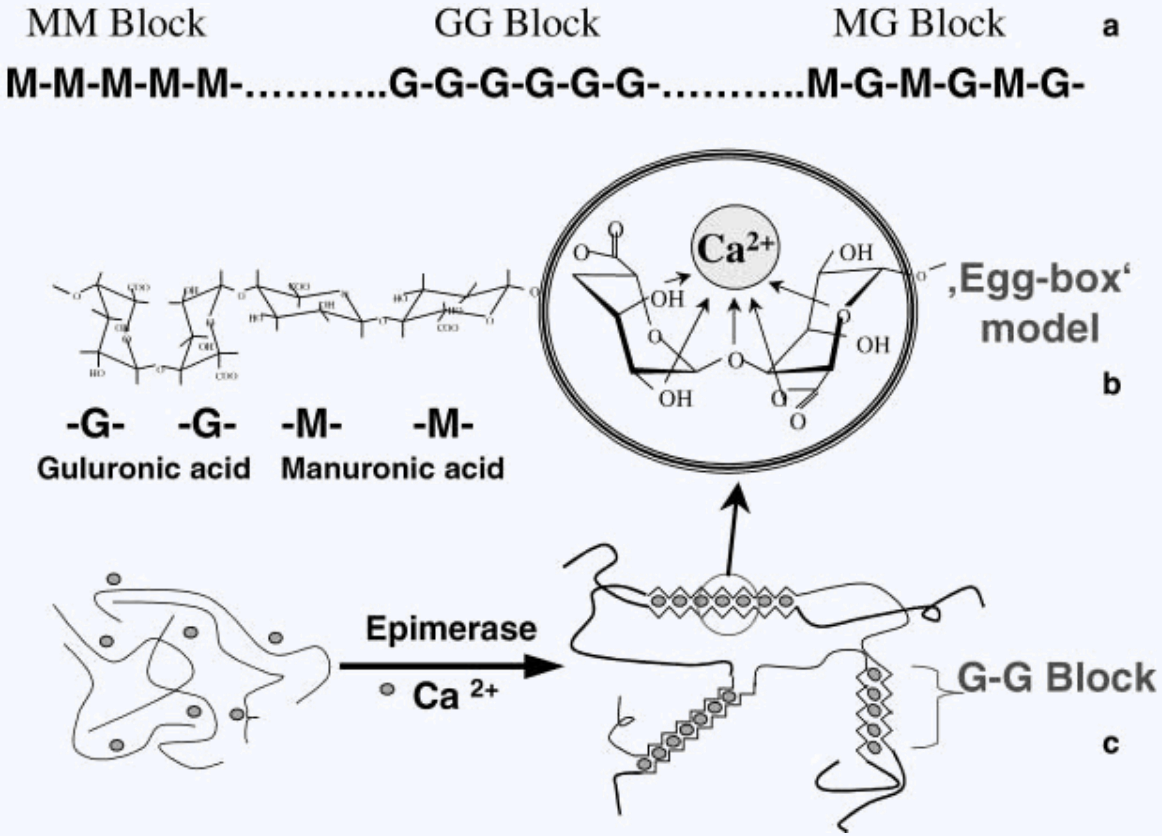
Alginate structure

Alginates: linear copolymers of blocks of **β-(1–4) linked d-mannuronic acid** and ***α*-(1–4)-linked L-guluronic acid** units



Alginate properties

The two monomers, D-mannuronic acid (M) and L-guluronic acid (G) arranged in an ‘egg-box’ structure, in which divalent ions (Ca^{2+}, Ba^{2+} or Sr^{2+}) intercalate.



Alginate properties

Alginate extraction

Found in brown seaweed

Global market of €400M
Estimates of about €1B by 2025

Worldwide seaweed industry provides total value of US$10 billion per year

Industrial production

Extraction of alginate from seaweed was first undertaken by Edward Stanford, a Scottish scientist, in 1883.

His patent explains how alginate can be extracted by soaking seaweed in water or dilute acid, extracting with sodium carbonate and then precipitating the alginate out of solution with acid.

1940s, and from the 1980s they found applications in wound dressing owing to their excellent gel-forming characteristics and also good hemostatic and absorbent properties

Industrial production

Sorbsan (introduced in 1983), first commercial alginate wound dressing
Kaltostat (early 1990s) wound dressing made of mixture of calcium and sodium alginate

Promotes healing by:

- Provides a moist environment
- Able to adsorb large amounts of extrudates
- Does not stick to the wound

Industrial production

Photos are meant to be representative of packaging/product you may receive if ordered. Appropriate product information will be printed on the respective labels. FMC reserves the right to change package types at its discretion.

PRONOVA SLG20

$199,00 / vial of 250 mg

Minimum order is one vial of 250mg

Grade GMP

1 ADD TO CART

SKU: Product# 4202006
Category: Sterile Ultrapure Alginates PRONOVA SL

Description Specifications Quality Safety & toxicology Application examples

Description

PRONOVA™ SLG20 is made from an alginate where over 60% of the monomer units are guluronate and is a highly purified and well-characterized sodium alginate. One of the functional properties of alginate is viscosity. The viscosity of an alginate solution can be manipulated by changing the concentration of the alginate or by using materials with varying chain length (i.e. molecular weight). Typically, the molecular weight for PRONOVA™ SLG20 is in the 75000 – 220000 g/mol range. The ultra low levels of endotoxins, proteins and product sterility allows for a big variety of in vitro and in vivo applications.

- Viscosity [mPa*s] : 20-99
- Appr. Mw [kDa] : 75-150
- G/M Ratio : ≥ 1.5
- Endotoxins [EU/g] : ≤ 100
- Total viable count [cfu/g] : Sterile

Industrial production

Alginate used for the encapsulation of drugs

Advantage:

- Diversity types of materials
- Negative charge that for interaction with loaded drugs

Type of alginate microspheres	Preparation technique	Type of biomacromolecule loaded	Application
Nano-in-micro Al microspheres	Microsphere droplet generator technique with LbL self-assembly of polyelectrolytes	Dexamethasone, diclofenac and enzyme GOx	Combined drug delivery with 'Smart Tattoo' glucose sensor implants, diabetes monitoring
Al microspheres	Al suspension	Doxorubicin, irinotecan	Intratumoral drug delivery for antiglioma activity
	Emulsion/external gelation with LbL nanofilm coatings, encapsulation into HA hydrogels	TGF-β3, human MSCs	Cartilage tissue engineering
	Water-in-oil emulsion	Chemokines; CCL21, CCL19, CXCL12, CXCL10	Chemoattractant delivery, chemotaxis studies
	Water-in-oil emulsion	Plasmid DNA	Oral DNA vaccine delivery
	Antigen encapsulation by ultrasonic nozzle spray, emulsion-cross-linking methods	Live porcine rotavirus, recombinant VP6 protein	Oral vaccine delivery to ruminants
TSP-Al mucoadhesive microspheres	Ionotropic gelation with $CaCl_2$	Gliclazide	Oral drug delivery for antidiabetic activity
Mucoadhesive Al microspheres	Emulsification phase separation method	Acyclovir	Gastroretentive drug delivery
Inhalable Al microparticles	Cross-linking Al in presence of elastin using Ca^{2+} and spray drying	Enzyme elastase	Enzyme-specific pulmonary delivery
Calcium-Al beads	Emulsion dehydration method	Diloxanide furoate	Colon-specific delivery for amoebiasis treatment
	Octodecanol, berberine in sodium-Al suspension dripped in $CaCl_2$	Berberine	Stomach-specific delivery
Coralline hydroxyapatite-Al composite microspheres	Dispersion polymerization technique	Antibiotic gentamicin	Bone repair Bone regeneration
Calcium titanium phosphate-Al and hydroxyapatite-Al microspheres	Preadsorption onto ceramic particles or dispersion in Al matrix	GCR enzyme	GD treatment, bone regeneration templates
Silicate-Al composite microspheres	Combining Ca-silicate shell with Al core	BSA	Bone tissue regeneration
Al-chitosan microspheres	Membrane emulsification in combination with Ca^{2+} and chitosan solidification	Insulin	Oral insulin delivery for diabetes
	Ternary complexation with Quality by design approach	Celecoxib	Colon-specific delivery
Al-chitosan-PCL microspheres	Emulsification method using $CaCl_2$	VEGF	VEGF delivery
PLL-Al microspheres	Simulated physiological compressive loading technique	VEGF	Delivery to HUVEC for promoting vascularization of tissue-engineered bone grafts
PLL-Al microparticles	Ionotropic gelation and cross-linking Al with Ca^{2+} ions and PLL	ASO	Intestinal delivery

Al: Alginate; ASO: Antisense oligonucleotides; BSA: Bovine serum albumin; CNT: Carbon nanotube, DDS: Drug delivery system; GCR: Glucocerebrosidas

Poojari, Radhika, and Rohit Srivastava. 2013. "Composite Alginate Microspheres as the next-Generation Egg-Box Carriers for Biomacromolecules Delivery." *Expert Opinion on Drug Delivery* 10 (8): 1061–76.

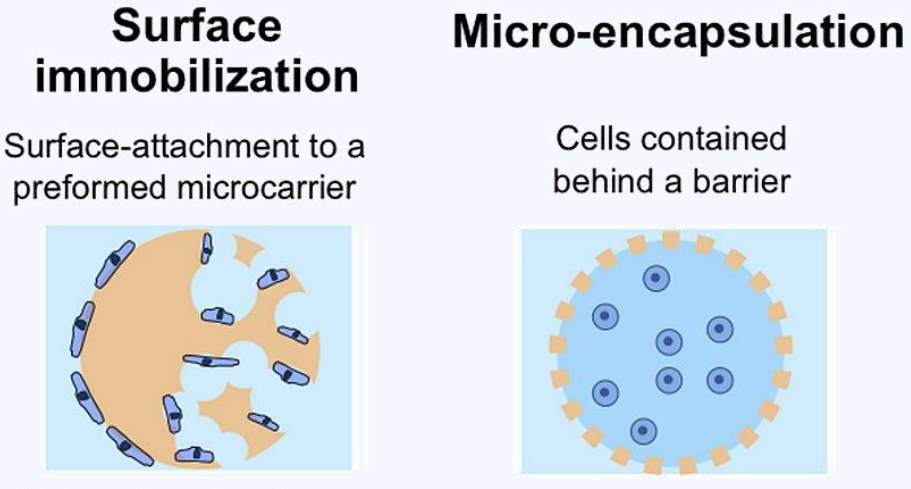
Industrial production

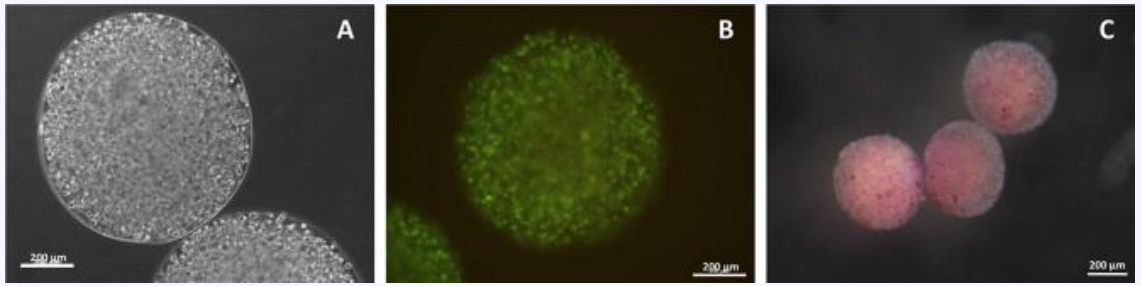
Encapsulation of cells in alginate matrix

- High viability
- Optimal porosity

But…

- Low bioactivity
- Fibrous encapsulation





Bidarra, Sílvia J., Cristina C. Barrias, and Pedro L. Granja. 2014. "Injectable Alginate Hydrogels for Cell Delivery in Tissue Engineering." *Acta Biomaterialia* 10 (4): 1646–62.

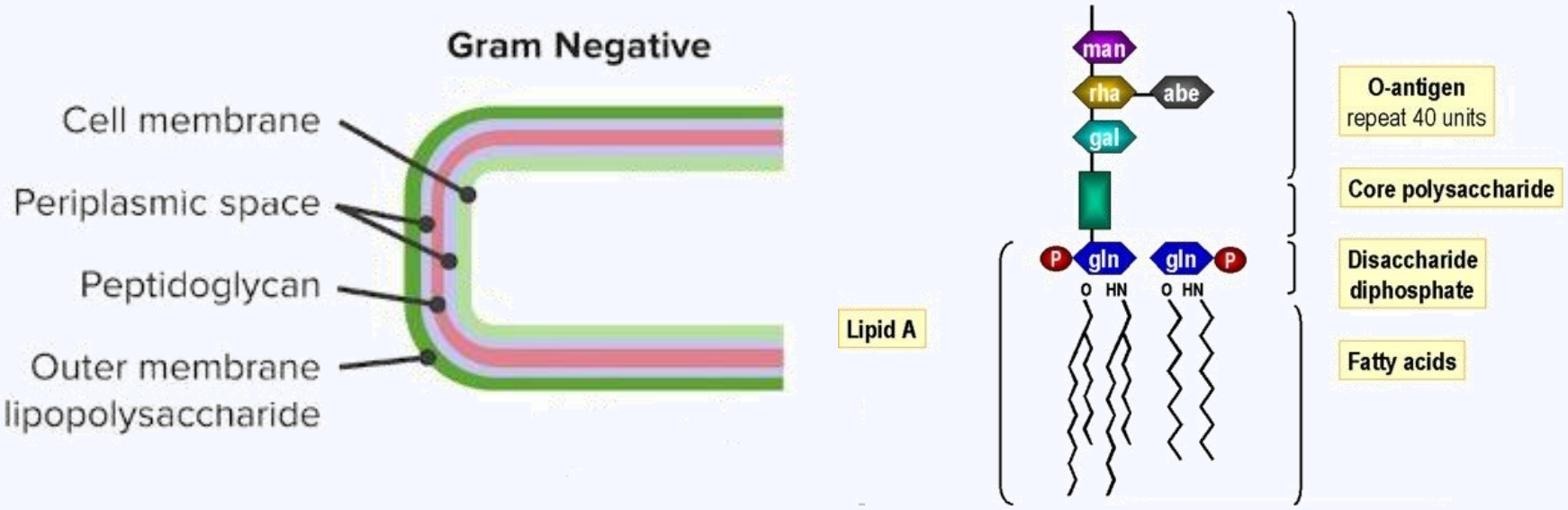
Limulus amebocyte lysate (LAL)

600,000+ horshoe crabs captured per year to donate around 30% of their blood

The blood is worth $60,000 a gallon in a global industry valued at $50 million a year.

LAL endotoxin test

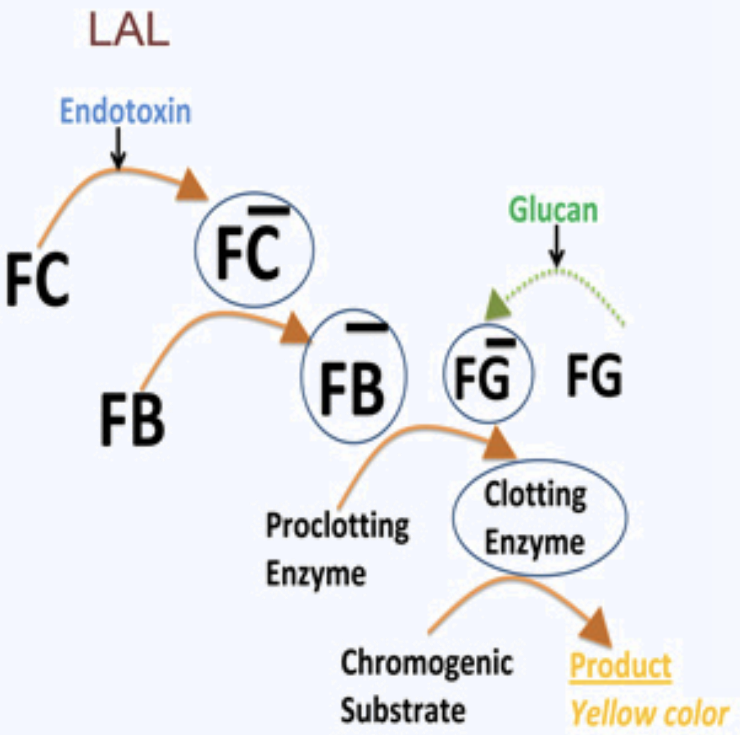
Lipopolysaccharide (LPS)



Responsible for toxic effects (fever and shock)

Activates macrophages that release inflammatory cytokines

LAL endotoxin test



Whatis the industry developing next?
Why?

Medical device vs drug

- Drugs: 12 years and near $1B
- Medical devices: 7 years and $10-100M

"Calcium alginate wound dressing are FDA approved"

Silverlon calcium alginate wound dressins, which consist of a sterile, non-woven pad composed of high M (mannuronic acid) alginate and a silver nylon contact layer, are FDA-approved for the following clinical indication: management of moderately to heavily exudating partial and full thickness wounds, including...

Combination products

Combination products are therapeutic and diagnostic products that combine drugs, devices, and/or biological products.

Examples of single-entity combination products:

- Monoclonal antibody combined with a therapeutic drug
- Device coated or impregnated with a drug or biologic: drug-eluting stent, pacing lead with steroid-coated tip, catheter with antimicrobial coating, condom with spermicide, transdermal patch